Study on yeast enzymatic activities by near infrared spectroscopy and radiorespirometry

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Introduction

In the field of yeast fermentation, optimisation of biotechnological processes requires the application of a rapid and accurate method which offers the possibility of a feedback quality control.

The reliability of NIR spectroscopy was supervised by using a radiorespirometer fitted with an ionisation chamber.[1] Our aim is to study the applicability of near infrared (NIR) spectroscopy to measure the yeast enzymatic activities during the fermentation.

Material and methods

Yeast strain: *Saccharomyces cerevisiae* BRAS 268 was used in top fermentation (10°P wort). Storage was at 4°C on wort–agar slants. During fermentation, the yield of yeast cells was determined by dry weight expressed in mg 100 mL^{-1} obtained at 50°C, *in vacuo*. The carbohydrate content was determined by the EBC method and expressed in g $100g^{-1}$.

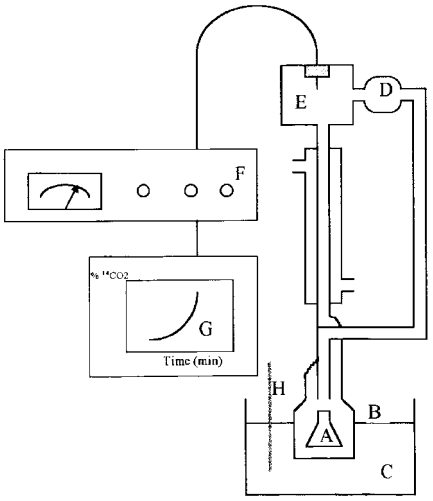

Figure 1. Scheme of radiorespirometry.[1]

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The production of yeast acetone powders was described elsewhere.[2] NIR spectra were recorded every 2 nm from 1100 to 2500 nm by using a spectrometer, PS Co 6250, working in a single beam mode. The active water content was determined by NIR spectroscopy and calculated by absorbance difference (log 1/*R*)—A: between 1938 and 1858 nm and —B: between 1938 and 2020 nm.

The enzymatic activities were obtained by radiorespirometry (Figure 1). The data were expressed in % of maximum degradation of glucose-U-^{14}C and initial velocity (*V*) of glucose-U-^{14}C degradation min^{-1}.

Results and discussion

The transformation of glucose by yeasts during fermentation has been carried out under proliferating conditions. Measurements were made at different time plots varying from 5 to 200 hour incubation. Fermentation profiles of the various carbohydrate contents and yeast production are presented in Figure 2.

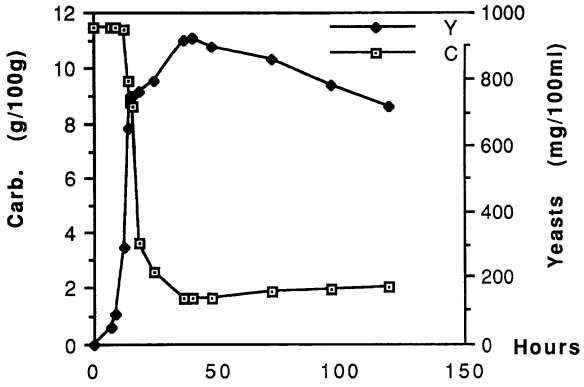

Figure 2. Carbohydrate content and yeast production.

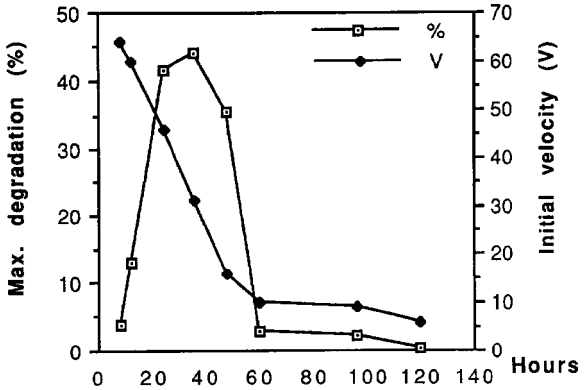

Figure 3. Initial velocity and degradation of glucose C14.

The results showed a linear relationship between, on the one hand, the breakdown of glucose-U-^{14}C and the enzymatic activities (Figure 3) and on the other hand, active water content in living yeast cells (Figure 4 and Figure 5).

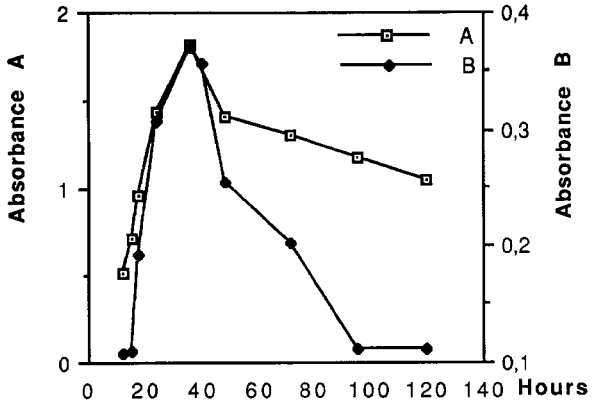

Figure 4. Active water contents during yeast fermentation.

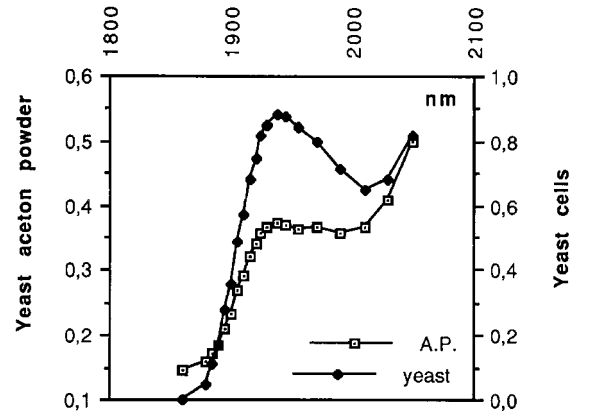

Figure 5. NIR spectra of yeast and yeast acetone powder.

Table 1. Maximum degradation (%$^{14}CO_2$) in different reaction systems.

Systems with	glucose 2-^{14}C	glucose 3,4-^{14}C
ATP = 0.01 mg	25	40
ATP = 0	0	0

Glucose 2-^{14}C or glucose 3,4-^{14}C, 1 micro Ci/nM; yeast acetone powder 50 mg; 1/15 M phosphate buffer 1 mL, pH 4.6; Temp. 28°C.

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Let us note, by using glucose-U-^{14}C and glucose-2-^{14}C (instead of glucose-U-^{14}C) that with yeast acetone powders, enzymes of Embden–Meyerhof–Parnas and tricarboxylic acid pathways were active only if ATP traces were added in the mixture reaction systems as described in Table 1.

Conclusion

These results show that NIR spectroscopy, with the help of radiorespirometry, could provide the possibility to rapidly follow any yeast fermentation process for qualitative and quantitative purposes.

References

1. J. Mayaudon, *Soil Biochemistry* **2,** 202 (1971).
2. I.C. Gunsalus, *Methods in Enzymology* **1,** 51 (1955).